COMPARISON OF PYROLYZATES FROM FERMENTED AND UNFERMENTED CIGAR FILLER TOBACCO

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The change in leaf constituents during fermentation of cigar tobacco has been the subject of numerous investigations. However, a comparison of pyrolyzates or smoke condensates of cigar tobacco before and after this process has not been reported. When unfermented cigar tobacco is burned, a pungent irritating smoke is produced. Fermentation causes the necessary change in the tobacco so that smoking produces a desirable combination of flavor and aroma. Comparison of the qualitative and quantitative changes in the volatile smoke components should, therefore, reveal some of the components responsible for this improvement.

The results of this preliminary study indicate that qualitative changes are minor; most quantitative changes are consistent with known alterations in leaf constituents during fermentation. The possibility cannot be ruled out that some qualitative changes may be occurring which are beyond the sensitivity of the techniques used, yet because of low threshold value for aroma and taste do play an important role.

INTRODUCTION

Fermentation of cigar filler tobacco is a necessary step in the manufacture of cigars. The unprocessed leaf produces a pungent, irritating smoke unacceptable to the consumer. Undesirable flavor and aroma components of this smoke apparently mask pleasurable effects due to other smoke components. Changes in leaf composition during fermentation appear to remove or reduce precursors of these undesirable components. At the same time desirable smoke components may be unmasked or increased.

The nature of the chemical conversions in the leaf during fermentation has been investigated in some detail by Frankenburg (3) and Vickery (13). However, the effect of these alterations on smoke composition or pyrolysis products has not been reported.

In recent years experiments have been conducted in this laboratory to evaluate precursor—product relationships between tobacco leaf components and smoke components. Since pyrolysis may be considered to be representative of processes occurring during smoking, it was felt that this technique could be used to examine the thermal decomposition of cigar filler tobacco before and after fermentation. Ideally, qualitative and quantitative changes in pyrolysis products might provide an index of the degree of fermentation and therefore the marketability of the product.

This preliminary study was designed to cover a broad area. We were particularly interested in whether large differences were present both qualitatively and quantitatively in the gas chromatographable components present in both pyrolyzates and distillates from fermented and unfermented tobacco. Smaller, more subtle

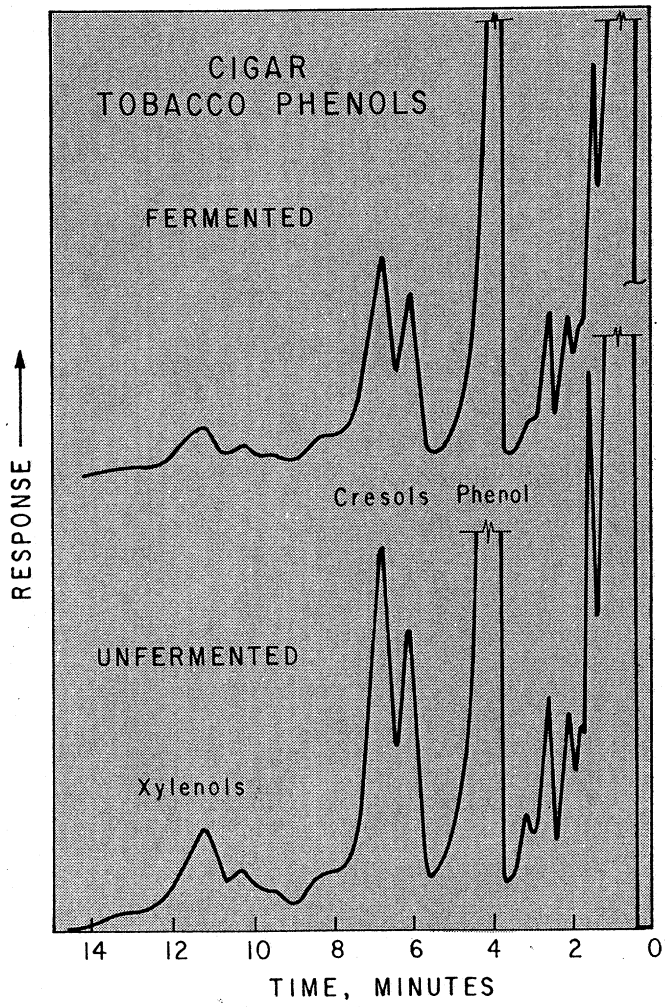

Figure 1—Gas chromatograms of the phenol fractions obtained from fermented and unfermented cigar filler tobacco pyrolyzed from ambient to 1000°C. Conditions of chromatography: Column—5' x 1/8" o.d. stainless steel containing 5% SE-30 on 80/100 Chromosorb Q. Temperature programmed from 60°C to 250°C at 6°/min. Helium flow 32 ml/min.

differences will be the subject of future studies.

MATERIALS AND METHODS

Preparation of Tobacco Samples. The tobacco utilized in this study was Pennsylvania cigar filler, 1963 crop, received directly from the manufacturer immediately before and after fermentation. All comparisons were made between tobacco samples from the same bulk.

Prior to pyrolysis, leaves were stripped of stems and large veins and cut into pieces approximately 1 cm^2. Samples were removed and dried to constant weight (80°C, 24 hours) to determine moisture content. Variations in moisture content were less than 0.5% between fermented and unfermented samples; therefore, equal weights of the various samples were used for comparative pyrolytic studies.

Pyrolytic Techniques. Pyrolysis was performed in an unobstructed quartz tube (4 ft × 12.5 cm) enclosed in a Lindberg Hevi-Duty furnace[1]. Temperature was controlled by a variable transformer and was measured by a platinel II thermocouple, located in the center (hot zone) of the furnace, in conjunction with a Leeds and Northrup Model 8692 potentiometer. The gaseous environment was dry nitrogen maintained at a flow rate of 30 ml/min. Initially experiments were performed by positioning the sample in the center of the quartz chamber and then inserting the chamber in the unheated furnace. In the first series of experiments, the temperature was raised from ambient to approximately 1000°C at a rate of 12°C/min. In the second series, the sample was positioned in the quartz tube prior to placing the tube and its contents into the preheated furnace. The latter series of experiments were performed at temperatures of 330°, 440°, 660°, and 850°C. In all cases products were collected by passing the resulting effluent through a series of three cold-finger traps (ice-water, dry ice-acetone) followed by a gas scrubber containing diethyl ether and 0.5% sodium hydroxide (1/1 v/v).

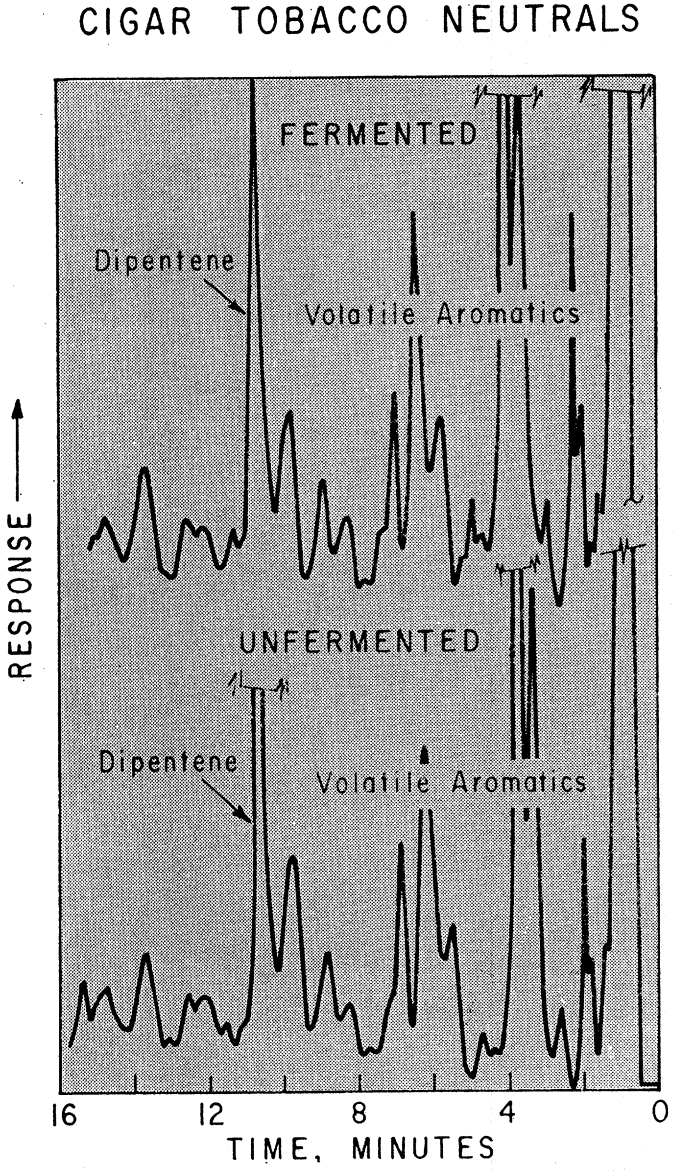

Figure 2—Gas chromatograms of the neutral fraction obtained from fermented and unfermented cigar filler tobacco pyrolyzed from ambient to 1000°C. Conditions of chromatography same as Figure 1 except column was run isothermally at 120°C.

Fractionation of the Pyrolyzate. The pyrolyzate was recovered from the cold traps by back washing with ether and sodium hydroxide from the gas-scrubber. The recovered pyrolyzate was then partitioned into an organic layer containing neutrals and bases, and an aqueous alkaline layer containing sodium salts of the phenols and carboxylic acids. By appropriate solvent extractions and pH adjustment (8-11) five principal fractions, suitable for analysis, were obtained: a) ether-soluble neutrals, b) ether-soluble bases, c) ether-soluble weak acids (phenols), d) ether-soluble carboxylic acids, and e) water-soluble carboxylic acids.

Analytical Methods. The neutral fractions (a) were analyzed on an Aerograph Model 1200 gas chromatograph with flame ionization detector (detector temperature 275°C) and equipped with a 5 ft. × ⅛ in. o.d. stainless steel column (5% SE-30 on 80/100 mesh Chromosorb Q) programmed from 60°C to 250°C at 6°C/min. with helium flow of 32 ml/min.

Phenolic fractions (c) were analyzed on the above column isothermally at 120°C.

Basic fractions (b) were analyzed on a 5 ft. × ⅛ in. o.d. stainless steel column (15% Carbowax on 60/80 Chromosorb W) under the same conditions of temperature programming and carrier flow as in the analysis of neutrals (a).

The ether- and water-soluble carboxylic acid fractions [(d) and (e) respectively] were analyzed by gas chromatography of their trimethyl-silyl ester derivatives by a modification of the method of Jones and Schmeltz (6). An Aerograph Model 1520 C with flame ionization detector was used. Separations were made on a 5 ft. × ⅛ in. o.d. stainless steel column (5% SE-30 on 60/80 Chromosorb W) programmed from 70°C to 240°C at 6°C/min. with a helium flow of 30 ml/min.

RESULTS AND DISCUSSION

The reduction of the harsh and undesirable flavor and aroma in cigar filler tobacco over the period of fermentation suggests that there are corresponding quantitative if not qualitative changes in the volatile smoke components. In attempting to identify such changes, one should consider two sources of flavor and aroma components (e.g. distillation products which volatilize behind the burning coal or pyrolysis products formed in or near the coal).

In this study we attempted to collect each type of thermally-released product using two different methods. In the first method, described in the methods section, we collected pyrolyzate from a programmed

[1] *Mention of commercial items does not imply their endorsement by the Department over similar products not mentioned.*

temperature run, over the range from ambient to 1000°C. The second method consisted of collecting and characterizing pyrolyzates from tobacco exposed to several different temperatures under isothermal conditions.

Each pyrolyzate was separated into the five principal fractions outlined in the methods section. Quantitative changes observed correlated with compositional changes of tobacco known to occur during fermentation and with the results of previous experiments involving pyrolysis of leaf components (1,4,5,8-11,14). No major qualitative changes were found in any of the comparable fractions examined.

Programmed temperature experiments. In samples from both the unfermented and fermented tobacco, two major regions of thermal degradation were observed during the programmed temperature experiments. A dark aerosol evolved in the range from 330°C to 550°C and a second, less intense, white aerosol appeared from 660°C to 800°C; the latter aerosol presumably arose from pyrolysis of the more stable leaf components. These observations are in agreement with previous studies (2,7) which demonstrated several regions of weight loss on thermal gravimetric analysis of tobacco.

Ether soluble bases in the pyrolyzate of unfermented tobacco consisted largely of distilled nicotine with smaller amounts of nicotine pyrolytic products; pyridine, methylpyridines, 3-vinyl-pyridine and 3-cyanopyridine. In addition, other alkaloids were present in smaller quantities. A substantially lower level of nicotine was observed in the comparable fraction derived from the pyrolyzate of fermented tobacco. There was also a correspondingly low level of the volatile pyridine bases. In addition the pyrolyzate of the fermented tobacco contained a substantially higher level of higher boiling alkaloids and various dehydrogenation and oxidation products of nicotine.

Volatile phenols were of special interest because of their organoleptic (12) and physiological (15) properties. Here again **(Figure 1)** the qualitative picture (pyrolyzate from fermented vs. unfermented) is similar; however, quantitatively there were substantially lower levels of phenols, cresols, and xylenols in the pyrolyzate from the fermented tobacco. Reductions were in the range of approximately 40%. This observation is consistent with the reduction of leaf carbohydrates and leaf polyphenols (both phenol precursors (1,8)) during fermentation.

The carboxylic acid fractions, both ether- and water-soluble, derived from the pyrolyzates of the fermented and unfermented tobaccos, were examined by gas chromatography of the trimethylsilyl esters of the acids. Many high and low boiling acids were present in the pyrolyzates from unfermented samples. Following fermentation there was generally a decrease in these acids. In many of the fractions, particularly in the water-soluble fraction, malic acid appeared to be the predominant component.

Comparison of the neutral fraction from pyrolyzates of both unfermented and fermented tobacco showed very little qualitative or quantitative differences. Each fraction contained a large number of volatile aromatics and the prominent smoke component, dipentene. The gas chromatograms of these fractions were comparable to those obtained in previous work (9) when the hexane-soluble or lipid fraction of the leaf was pyrolyzed. That lipids are resistant to change during fermentation (3,13) is corroborated by the similarity of gas-chromatographic patterns apparent in **Figure 2.**

Isothermal experiments. In these experiments exposure of both the fermented and unfermented tobaccos to lower temperatures resulted generally in distillation of volatile leaf components. On the other hand, at the higher temperatures, pyrolyzates contained increasing amounts of newly formed substances in addition to the distillates.

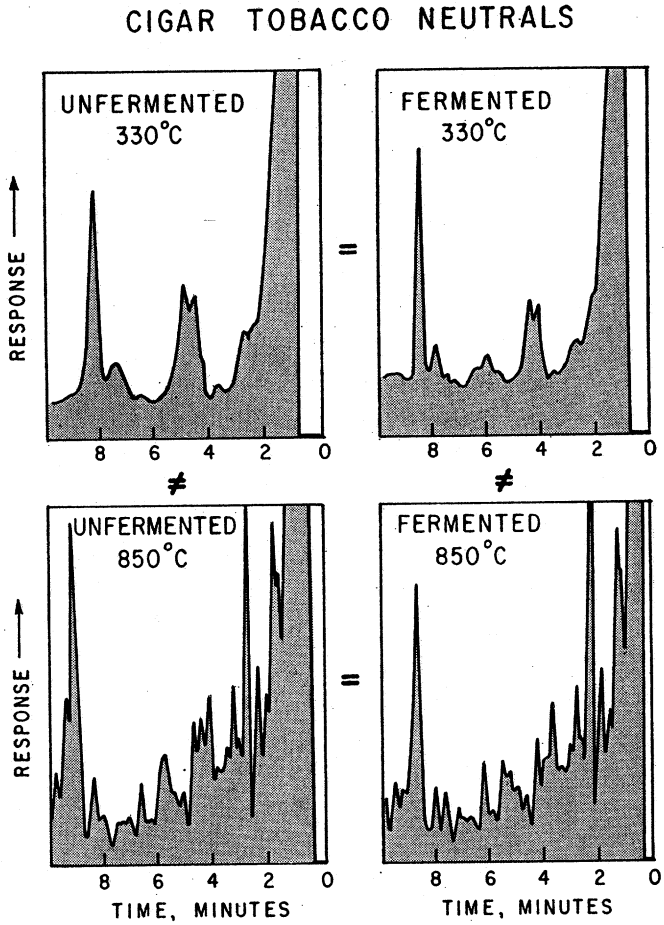

Figure 3—Gas chromatograms of the neutral fractions obtained from fermented and unfermented cigar filler tobacco pyrolyzed at constant temperatures. Conditions of chromatography same as Figure 1.

Comparable fractions from the pyrolyzates of both fermented and unfermented tobacco were qualitatively distinct at each temperature. At each individual temperature the pyrolyzates from both fermented and unfermented tobacco were quite similar. This is illustrated in **Figure 3.** In spite of the distinct qualitative difference evident between the neutrals at 330°C and 850°C there is very little difference between fermented and unfermented samples at the same temperature. The volatile components observed at each temperature reflected the different thermolytic mechanisms involved. This same relationship between comparable fractions of the isothermally produced distillates and pyrolyzates held true for all the other fractions examined.

CONCLUSION

We have attempted to determine differences in volatile smoke components which might reflect the change in flavor and aroma which accompanies the fermentation process. Utilizing the techniques of pyrolysis and gas chromatography we did not observe any large qualitative differences. Several quantitative differences were noted which were consistent with known changes in leaf composition during fermentation.

The results indicate that the improvement in taste and aroma arising from fermentation could be the

result of quantitative changes in smoke composition. They do not rule out the possibility that minor or undetected qualitative changes may be responsible. While we have not determined the precise chemical source of the improvement in cigar tobacco quality during fermentation we feel that these methods will ultimately be able to do so.

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